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Epidermal growth factor receptor tyrosine kinase inhibition is not protective in PCK rats

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Background. Advances in the understanding of cystogenesis, identification of the *PKHD1* gene and availability of a rat model (the PCK rat) caused by a *Pkhd1* mutation facilitate testing of therapies for autosomal-recessive polycystic kidney disease (ARPKD). Considerable support exists for the importance of the epidermal growth factor (EGF)/transforming growth factor-alpha (TGF-α)/EGF receptor (EGFR) axis and of the adenylyl cyclase-adenosine 3′,5′-cyclic monophosphate (cAMP) pathway in the pathogenesis of cyst formation and progressive enlargement.

Methods. To determine whether EGFR tyrosine kinase inhibition is protective in the PCK rat, male and female animals were treated with EKI-785 or EKB-569 or with vehicle alone between 3 and 10 weeks of age. Biochemical and histomorphometric analysis, immunohistochemistry, immunoblotting, enzyme immunoassay, and quantitative reverse transcription-polymerase chain reaction (RT-PCR) were used to ascertain the effects of treatment.

Results. Contrary to other murine models of ARPKD, overexpression and apical mislocalization of EGFR were not detected in the PCK rats. Consistent with these expression results, EKI-785 or EKB-569 administration had no effect or worsened PKD, and had no effect on the development of fibrocystic liver disease. Increased renal cAMP and vasopressin V2 receptor expression were observed in the EKI-785–treated animals.

Conclusion. EGFR tyrosine kinase inhibition did not protect PCK rats from the development of PKD. This may be due to effects on collecting duct cAMP that counteract possible beneficial effects on the extracellular-regulated protein kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway, particularly in the absence of EGFR overexpression or mislocalization. The relevance of these observations to the treatment of human cystic kidney diseases deserves further study.

Key words: polycystic kidney disease, PCK rat, EGF receptor tyrosine kinase inhibitor.

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Autosomal-recessive polycystic kidney disease (ARPKD) is an important childhood nephropathy that results in death in 30% of affected infants and end-stage renal disease (ESRD) during the first decade of life in 50% of affected individuals who survive the neonatal period [1–4]. It is accompanied by biliary dysgenesis, which is often the major clinical feature in older patients [1–4]. It is characterized by fusiform dilation of the collecting ducts in the kidney and by proliferation and dilatation of the interlobular bile ducts and portal fibrosis in the liver. Only symptomatic treatment is currently available. Advances in understanding of cystogenesis [5–8], identification of the *PKHD1* gene [9, 10], and availability of an animal model orthologous to human ARPKD [11, 12] may facilitate the development of therapies. Increasing evidence supports the importance of the epidermal growth factor (EGF)/transforming growth factor-alpha (TGF-α)/EGF receptor (EGFR) axis [13–17] and of the adenylyl cyclase-adenosine 3′,5′-cyclic monophosphate (cAMP) [18–23] pathway in promoting renal tubular epithelial cell proliferation and cyst formation. Consequently, interventions aimed at blocking these pathways have been tested in animal models of polycystic kidney disease (PKD). Administration of a vasopressin V2 receptor antagonist lowered renal cAMP and markedly inhibited cystogenesis in three models of slowly progressive PKD (pcy mouse, orthologous to adolescent nephronophthisis; PCK rat, orthologous to human ARPKD, and *Pkd2*$^{WS25/-}$ mouse, orthologous to human ADPKD) [24, 25] and is also effective in the cpk mouse, a model of rapidly progressive PKD [26]. Genetic and/or pharmacologic inhibition of EGFR tyrosine kinase activity markedly inhibited cystic disease development [14, 17] in bpk and orpk mice, two models of autosomal-recessive, rapidly progressive renal cystic disease, and in Han:SPRD rats [27], a model of autosomal-dominant slowly progressive renal cystic disease. Finally, administration of an inhibitor of the tumor necrosis factor-α (TNF-α) converting enzyme, a metalloproteinase that cleaves and releases the EGFR

ligand TGF-α, reduced the severity of cystic disease in bpk mice [28], particularly if combined with inhibition of EGFR tyrosine kinase activity [29]. The purpose of this study was to determine whether EGFR tyrosine kinase inhibition is effective in the PCK rat, a model of human ARPKD.

METHODS

EGFR tyrosine kinase inhibition

EKI-785 is a small molecule inhibitor specific for EGFR tyrosine kinase [30]. This compound binds irreversibly to the ATP binding site of EGFR, inhibits the kinase activity of this protein, blocks EGFR autophosphorylation, inhibits cell proliferation, and blocks the growth of tumors that overexpress EGFR. It also inhibits Erb-B2 activity. The duration of EKI-785 activity is dependent on the half-life of the compound and the turnover rate of EKI-785–bound EGFR in the plasma membrane. Preliminary experiments demonstrated that administration of EKI-785 intraperitoneally every 3 days resulted in optimal inhibition with minimal toxicity. EKB-569 is a derivative of EKI-785 with improved pharmacokinetics and enteral bioavailability compared to the parent compound [31].

Experimental animals and study design

Male and female PCK rats maintained at the animal facilities of the Mayo Clinic, Rochester, Minnesota, were used for three experimental protocols.

Intraperitoneal administration of EKI-785. At 3 weeks of age, male PCK littermates and wild-type Sprague-Dawley rats were divided into two groups. One group was injected intraperitoneally with 90 mg/kg of EKI-785 every 3 days, between 3 and 10 weeks of age. This dosage was based on previous pharmacodynamic studies of this compound in other rat strains. The other group of animals received vehicle alone (2% Tween 80 and 0.5% methylcellulose in water). The rats were given a standard rat laboratory diet and water ad libitum.

Intraperitoneal administration of EKB-569. At 3 weeks of age, male and female PCK littermates and wild-type Sprague-Dawley rats were divided into two groups. One group was injected intraperitoneally with 20 mg/kg of EKB-569 every 3 days between 3 and 10 weeks of age. This dosage was based on previous pharmacodynamic studies of this compound in other rat strains. The other group of animals was injected with vehicle alone (2% Tween 80 and 0.5% methylcellulose). The rats were given a standard rat laboratory diet and water ad libitum.

Enteral administration of EKB-569. At 3 weeks of age male and female PCK littermates and wild-type Sprague-Dawley rats were divided into one control and three experimental groups. The experimental groups were administered 5, 10, or 20 mg/kg of EKB-569 by gavage daily, using a 24 gauge, 1 inch, stainless steel fitting tube with 1.25 mm bulb tip between 3 and 10 weeks of age. The control group received vehicle (2% Tween 80 and 0.5% methylcellulose) alone. The rats were maintained on a standard laboratory rat diet and water ad libitum.

Experimental protocol

At 10 weeks of age, the animals were weighed and anesthetized with ketamine 60 mg/kg and xylazine 10 mg/kg, intraperitoneally. Blood was obtained by cardiac puncture for determination of serum creatinine and blood urea nitrogen (BUN) levels. The right kidney, liver, and spleen were placed into preweighed vials containing 4% paraformaldehyde or 10% formaldehyde in phosphate buffer (pH 7.4). The tissues were embedded in Immunobed™ plastic embedding medium (Polysciences, Worthington, PA, USA) or in paraffin for histologic studies. The left kidney was immediately frozen in liquid nitrogen for Western blotting analysis and determination of tyrosine kinase activity.

Histomorphometric analysis and immunohistology

For light microscopy, 5 μm transverse tissue sections, including cortex, medulla, and papilla, were stained with hematoxylin-eosin and picrosirius red. Whole transverse tissue sections stained with hematoxylin-eosin were used to measure cyst volumes. Renal fibrosis was scored using the picrosirius red staining of collagen fibers. Image analysis procedures were performed with Meta-Morph software (Universal Imaging, West Chester, PA, USA). The MetaMorph software system includes a light microscope with a color digital camera (Nikon DXM 1200) and a Pentium IBM-compatible computer (Del OptiPlex). Stained sections were visualized under a Nikon microscope and digital images were acquired using a high-resolution Nikon digital camera and displaced on the monitor. Interactively applied techniques of enhancement were used for a better definition of interested structures and to exclude fields too damaged to be analyzed. A colored threshold was applied at a level that separates cysts from non-cystic tissue and picrosirius red–positive material from background in order to calculate the volumes of cysts or fibrosis. The areas of interest were expressed as a percentage of total tissue. Because of day-to-day variability in the intensity of staining, the processing for picrosirius red staining of all tissue sections from the same experiment was always done at the same time. Immunohistochemical staining of EGFR was conducted using rabbit polyclonal anti-EGFR antibodies (Cell Signaling

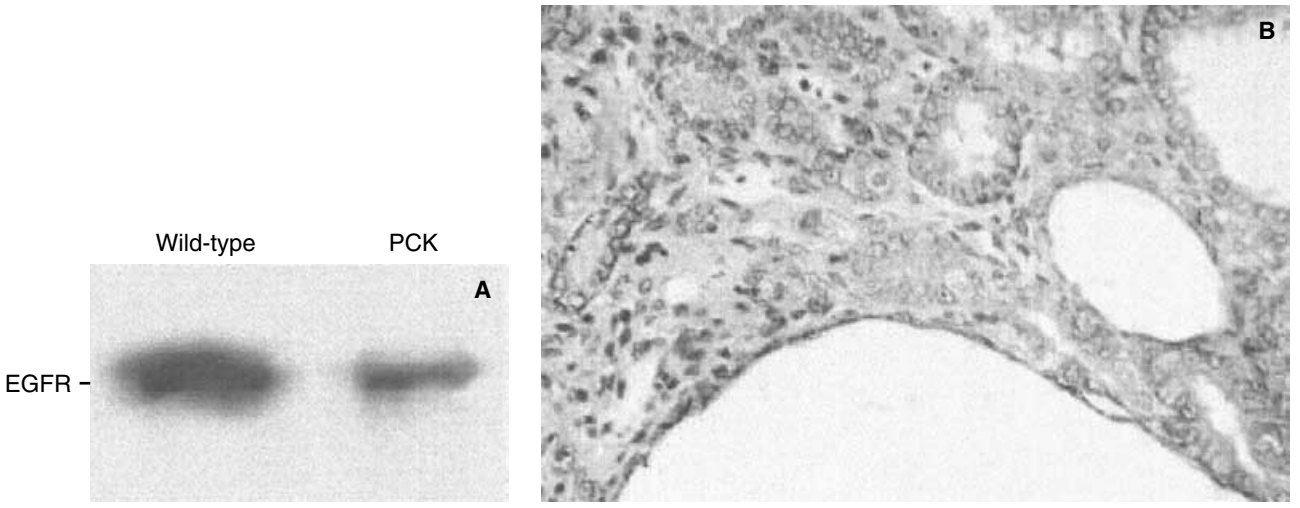

Fig. 1. Epidermal growth factor receptor (EGFR) expression analyzed by Western analysis and immunolocalization. (*A*) Immunoblot of proteins isolated from wild-type and PCK kidneys showing a lower EGFR expression in the cystic kidney. (*B*) Localization, mostly basolateral, of EGFR in tubules and cysts from PCK kidney.

Technology, Beverly, MA, USA) (catalog number 2232), as previously described [15].

Western blotting analysis

Protein was isolated from whole kidneys by homogenization in radioimmunoprecipitation assay (RIPA) buffer with protease inhibitors (complete minitablet; Roche Diagnostics, Mannheim, Germany). For Western blotting, 30 μg of total protein lysate were diluted in sodium dodecyl sulfate (SDS)–reducing buffer (62.5 mmol/L Tris-HCl, pH 6.8; 25% vol/vol glycerol; 2% wt/vol SDS; 0.01% wt/vol bromophenol blue; and 5% vol/vol p-mercaptoethanol) and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12% separating gel. Samples were transferred to a nitrocellulose membrane, treated with blocking buffer (5% dry milk and 0.05% Tween 20) and probed with rabbit polyclonal anti-EGFR antibody. Membranes were washed, probed with peroxidase-conjugated antirabbit immunoglobulin, treated with enhanced chemiluminescence (ECL) reagent (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and exposed to autoradiography film.

cAMP content of whole kidneys

Half kidneys rapidly frozen in liquid nitrogen were ground to a fine powder under liquid nitrogen in a stainless steel mortar and homogenized in 10 volumes of cold 5% trichloroacetic acid (TCA) in a glass-Teflon tissue grinder. After centrifugation at 600*g* for 10 minutes, the supernatants were extracted with 3 volumes of water-saturated ether. After drying the aqueous extracts, the reconstituted samples were processed without acetylation using the enzyme immunoassay kit from Sigma (Sigma-Aldrich, Inc., St. Louis MO, USA). The results were expressed in pmol/mg of wet tissue [32].

V2 receptor mRNA expression analysis by real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from rat kidney tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Four micrograms of total RNA were reverse transcribed using SuperScript First-Strand Synthesis System in a total volume of 50 μL (Invitrogen) at 37°C for 1 hour to synthesize cDNA. The amount of V2 mRNA per kidney specimen was quantified using the real-time PCR SYBR Green assay. The 50 μL PCR reaction contained 2 μL of diluted (50-fold) cDNA, 1× SRBRGreen JumpStart Tag ReadyMix (Sigma), and 200 nmol/L V2 and ribosomal protein L32-specific forward and reverse primers(V2 forward 5′-TCGTGCCTGTCAGGTTCTTATC-3′; V2 reverse 5′-TCGGATGGCCCTGGC-3′; ribosomal protein L32 forward 5′-GAAACTGGCGGAAACCCA-3′; and ribosomal protein L32 reverse 5′-GGATCTGGCCCTTG AATCTTC-3′) [33]. PCR reactions were performed in the DNA Engine Opticon System (MJ Research, Carlsbad, CA, USA) with 40 cycles at 95°C for 40 seconds and 60°C for 1 minute. All data were represented as relative to the ribosomal protein L32 level.

Statistical analysis

Comparisons between groups were made using one-way or two-way analysis of variance (ANOVA) with least significant difference comparisons of the means or Student *t* test as appropriate.

Table 1. Intraperitoneal administration of EKI-785 to male wild-type and PCK rats

	Sprague-Dawley+/+			PCK		
EKI-785 (mg/kg body weight)	Control ($N = 11$)	90 ($N = 7$)	P value	Control ($N = 7$)	90 ($N = 7$)	P value
Body weight *g*	332.6 ± 22.1	321.7 ± 27.6	0.427	364.6 ± 36.0	317.9 ± 18.3	0.107
Kidney weight *% body weight*	0.82 ± 0.04	0.85 ± 0.03	0.090	1.46 ± 0.30	2.79 ± 0.09	0.005
Liver weight *% body weight*	4.23 ± 0.17	4.42 ± 0.29	0.115	5.47 ± 0.42	6.17 ± 0.47	0.019
Spleen weight *% body weight*	0.38 ± 0.09	0.55 ± 0.15	0.013	0.32 ± 0.03	0.59 ± 0.15	0.001
Serum creatinine *mg/dL*	0.42 ± 0.04	0.40 ± 0.00	0.233	040 ± 0.00	0.51 ± 0.08	0.006
Serum blood urea nitrogen *mg/dL*	20.3 ± 2.5	22.7 ± 4.1	0.177	17.7 ± 1.8	30.6 ± 7.4	0.001
Kidney cyst volume %				20.8 ± 5.8	35.7 ± 5.8	0.001
Kidney fibrosis score %				2.39 ± 0.32	4.51 ± 1.54	0.001
Liver cyst volume %				6.02 ± 0.93	4.89 ± 1.11	0.077
Liver fibrosis score %				5.63 ± 0.83	5.09 ± 1.69	0.503

RESULTS

Renal expression of EGFR in PCK rats

The total EGFR levels measured by Western analysis were reduced in PCK rats as compared to the wild-type Sprague-Dawley rats (Fig. 1A). Consistent with these results, the EGFR staining of the kidneys in the PCK rats was weak and predominantly basolateral (Fig. 1B).

Intraperitoneal administration of EKI-785 and EKB-569

The intraperitoneal administration of EKI-785 significantly worsened the development of renal cystic disease, as reflected by higher kidney weights, concentrations of serum creatinine and BUN, cyst volumes and fibrosis scores (Table 1) (Fig. 2). It also caused a significant increase in the weights of the liver and spleen, without increasing the severity of the fibrocystic liver disease as reflected by similar hepatic cyst volumes and fibrosis scores in the EKI-785–treated and control rats. The higher weight of the spleens in the treated rats was unrelated to the fibrocystic liver disease, since it was also observed in the wild-type animals treated with EKI-785. The intraperitoneal administration of EKB-569 to male and female PCK rats had similar effects to that of EKI-785 to male rats (Table 2). Independent of treatment, the female PCK rats had more severe fibrocystic liver disease than their male counterparts (Table 2).

Enteral administration of EKB-569

The administration of EKB-569 by gavage at a dose of 20 mg/kg body weight every day resulted in a slight increase in the renal cyst volume without a significant change in kidney weight (Table 3). Lower doses did not have a significant effect on the kidneys. The administration of EKB-569 at doses of 10 or 20 mg/kg body weight every day resulted in significantly higher liver and spleen weights without an aggravation of the fibropolycystic liver disease as reflected by the histopathologic scores.

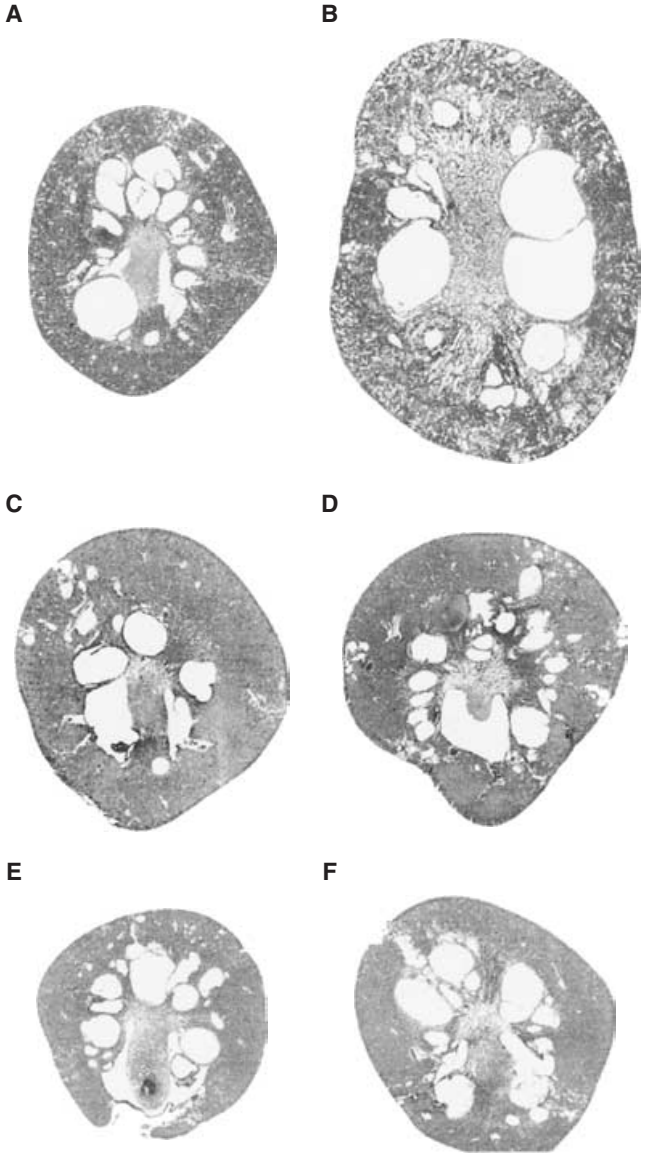

Fig. 2. Representative cross-sections (hematoxylin-eosin) of kidneys from control PCK rats (*A*, *C*, and *E*) and PCK rats treated with intraperitoneal EKI-785 (*B*) or EKB-569 (*D* and *F*). Kidneys (A to D) are from male animals, and kidneys (E and F) are from female animals.

Table 2. Intraperitoneal administration of EKB-569 to PCK rats

EKB-569 *mg/kg body weight*	Male		Female		*P* value	
	Control ($N = 9$)	20 ($N = 5$)	Control ($N = 8$)	20 ($N = 5$)	Gender	EKB20
Body weight *g*	299.4 ± 33.9	290.0 ± 6.7	214.3 ± 25.9	197.0 ± 8.3	<0.001	0.193
Kidney weight *% body weight*	1.52 ± 0.22	1.60 ± 0.10	1.27 ± 0.14	1.69 ± 0.33	0.346	0.006
Liver weight *% body weight*	4.52 ± 0.31	4.99 ± 0.26	5.30 ± 0.64	5.90 ± 0.69	<0.001	0.014
Spleen weight *% body weight*	0.34 ± 0.03	0.46 ± 0.07	0.38 ± 0.05	0.52 ± 0.08	<0.001	0.033
Serum creatinine *mg/dL*	0.50 ± 0.00	0.62 ± 0.28	0.50 ± 0.00	0.44 ± 0.05	0.070	0.532
Serum blood urea nitrogen *mg/dL*	17.9 ± 4.1	22.2 ± 7.6	15.6 ± 2.3	33.2 ± 22.5	0.299	0.013
Kidney cyst volume %	22.6 ± 8.1	24.3 ± 5.2	21.6 ± 3.3	27.9 ± 3.6	0.526	0.018
Kidney fibrosis score %	1.07 ± 0.46	1.73 ± 0.80	1.09 ± 0.49	1.93 ± 0.55	0.625	0.003
Liver cyst volume %	6.45 ± 1.40	4.89 ± 0.96	6.52 ± 1.25	7.74 ± 1.31	0.009	0.740
Liver fibrosis score %	7.30 ± 1.49	7.56 ± 1.95	7.94 ± 1.81	8.96 ± 1.25	0.133	0.339

Renal levels of cAMP and expression of V2 receptors

The renal concentrations of cAMP were significantly higher in the PCK than in the wild-type rats (Fig. 3) ($P < 0.001$). Since cAMP is mostly intracellular and its concentration in cyst fluid is very low [32], the cAMP accumulation in total kidney tissue likely underestimates that in the noncystic parenchyma. The intraperitoneal administration of EKI-785 to PCK rats was accompanied by a significantly higher renal accumulation of cAMP, as well as by a higher expression of the vasopressin V2 receptor (Fig. 3). The administration of EKB-569 had similar effects that did not reach statistical significance ($P = 0.13$ and $P = 0.14$, respectively).

DISCUSSION

This study shows that two irreversible EGFR tyrosine kinase inhibitors provide no protective effect and may worsen PKD in the PCK rat. This result is contrary to a large body of data supporting the importance of the EGF/TGF-α/EGFR pathway in the pathogenesis of PKD. Renal cyst fluids from ADPKD, ARPKD, and rodent models of PKD contain EGF-like peptides in mitogenic concentrations [34, 35], although the renal expression of EGF seems to be down-regulated [36–39]. TGF-α is over-expressed in ADPKD kidneys [40, 41]. EGF and TGF-α are cystogenic in in vitro systems [16, 42, 43]. Transgenic over-expression of TGF-α [44], Erb-B2 [45] (a member of the EGFR-related tyrosine kinase receptor family) and T24ras [46] (a transduction molecule in the EGFR signaling pathway) induce cystogenesis in mice. EGFR and Erb-B2 [13, 15, 47, 48] are overexpressed and mislocalized to the apical membrane of the cystic epithelium in human autosomal-dominant polycystic disease (ADPKD) and ARPKD and in rodent models of these diseases. The mislocalized receptors can bind EGF with high affinity, undergo autophosphorylation, and initiate a signaling cascade that results in cell proliferation [15]. Finally, genetic and/or pharmacologic inhibition of EGFR tyrosine kinase activity in vitro and/or in vivo inhibits the development of cystic disease in bpk and orpk mice [14, 17, 29] and in Han:SPRD rats [27].

The detrimental effect of EKI-785 and EKB 569 in this study is consistent with the low expression and the normal location of EGFRs in the PCK collecting tubule and cyst lining epithelial cells. Previous studies have shown that the mitogenic effect of EGF correlates with EGFR density [49]. The contrast in EGFR expression between the PCK rat and human ARPKD or murine models of human ARPKD remains unexplained. Possibly, the PCK rat is not a model for severe human ARPKD, despite being caused by a mutation in the rat ortholog *Pkhd1*. Similar to human ARPKD, the PCK rat is characterized by cysts in the collecting ducts and by bile duct dilatation and portal fibrosis [11, 12]. Nevertheless, the renal cysts develop later and predominantly in the outer medulla, and the degree of portal fibrosis is mild without formation of fibrous septa between portal tracts. Whether these differences are due to a hypomorphic mutation in the PCK rat, that does not completely inactivate the gene, or to species differences, genetic modifiers or other factors will require further study. These results may also reflect the pleiomorphic effects that EGF signaling and its inhibition may have in different model systems. Previous studies have shown that the role of EGF in cystogenesis may depend on the stage of the development [50], and that administration of EGF during the neonatal period (days 1 to 9, a period critical for the differentiation of the collecting duct) retarded the development of azotemia, common bile duct dilation, and intrahepatic bile duct proliferation in the bpk mouse [50] and renal cystogenesis and azotemia in the cpk mouse [51].

Despite the limitations that the PCK rat may have as a model of severe ARPKD, the observations in this study may be relevant to the treatment of slowly progressive forms of PKD. Cross-talk between cellular signal transduction pathways that involve tyrosine phosphorylation, such as the EGF/TGF-α/EGFR axis, and those mediated by G proteins, such as G protein coupled receptor induced

Table 3. Enteral administration of EKB-569 to PCK rats

EKB-569 *mg/kg body weight*	Male				Females				*P* value			
	Control ($N = 10$)	5 ($N = 9$)	10 ($N = 11$)	20 ($N = 11$)	Control ($N = 10$)	5 ($N = 11$)	10 ($N = 11$)	20 ($N = 10$)	Gender	EKB-5	EKB-10	EKB-20
Body weight *g*	350.9 ± 16.4	356.9 ± 26.0	337.5 ± 40.3	341.6 ± 25.4	233.8 ± 24.7	241.3 ± 20.1	237.1 ± 14.4	217.6 ± 32.5	<0.001	0.340	0.535	0.116
Kidney weight *% body weight*	1.61 ± 0.42	1.59 ± 0.25	1.60 ± 0.30	1.66 ± 0.23	1.36 ± 0.20	1.39 ± 0.18	1.34 ± 0.17	1.50 ± 0.09	<0.001	0.955	0.867	0.254
Liver weight *% body weight*	5.12 ± 0.30	5.18 ± 0.35	5.43 ± 0.68	5.50 ± 0.26	5.84 ± 0.31	6.10 ± 0.35	6.22 ± 0.26	6.56 ± 0.88	<0.001	0.133	0.013	0.002
Spleen weight *% body weight*	0.32 ± 0.03	0.34 ± 0.04	0.37 ± 0.09	0.36 ± 0.02	0.36 ± 0.03	0.39 ± 0.07	0.37 ± 0.04	0.39 ± 0.09	0.021	0.099	0.083	0.032
Serum creatinine *mg/dL*	0.61 ± 0.10	0.64 ± 0.05	0.57 ± 0.05	0.60 ± 0.04	0.59 ± 0.03	0.62 ± 0.07	0.57 ± 0.05	0.57 ± 0.07	0.195	0.173	0.128	0.468
Serum blood urea nitrogen *mg/dL*	19.0 ± 4.5	18.2 ± 2.1	18.7 ± 1.1	17.9 ± 2.3	16.6 ± 2.1	16.3 ± 2.2	16.5 ± 1.8	17.6 ± 5.0	0.009	0.803	0.802	0.983
Kidney cyst volume %	29.0 ± 12.7	33.2 ± 3.2	34.8 ± 10.1	34.9 ± 2.6	27.5 ± 10.0	30.7 ± 9.7	26.7 ± 10.8	34.3 ± 3.6	0.110	0.249	0.469	0.019
Kidney fibrosis score %	1.04 ± 1.27	1.01 ± 0.83	1.53 ± 0.71	1.24 ± 0.39	1.03 ± 1.07	1.09 ± 0.78	1.19 ± 0.75	1.12 ± 0.64	0.601	0.964	0.283	0.610
Liver cyst score %	5.58 ± 1.98	5.82 ± 1.86	4.40 ± 1.63	4.21 ± 0.96	5.75 ± 1.80	5.94 ± 2.39	5.61 ± 1.02	5.54 ± 2.13	0.077	0.746	0.199	0.159
Liver fibrosis score %	5.22 ± 1.54	4.19 ± 1.96	4.51 ± 1.31	3.96 ± 0.75	5.07 ± 1.25	4.67 ± 1.31	4.70 ± 1.00	4.67 ± 0.44	0.268	0.150	0.181	0.018

accumulation of cAMP, is complex and cell-specific. EGF and cAMP exert opposing proliferative and antiproliferative effects, respectively, on cultured normal human kidney cortex (NHK) cells [20]. On the other hand, they have complementary and additive proliferative effects on ADPKD cells [22]. It is uncertain whether this synergistic interaction also occurs in vivo. The results of this study suggest that EGFR tyrosine kinase inhibition in the PCK rat may have the unexpected effect of increasing the renal accumulation of cAMP.

The increased levels of cAMP and the up-regulation of the vasopressin V2 receptors in the kidneys of EKI-785–treated PCK rats and the detrimental effect of EGFR tyrosine kinase inhibition may be explained by a number of previous observations. EGFR tyrosine kinase activation triggers numerous downstream signaling pathways, including phosphorylation of G proteins, activation of phospholipase C, and downstream calcium and protein kinase C (PKC)-mediated cascades, and stimulation of the ERK pathway [52]. Purified EGFR can phosphorylate $G_{s\alpha}$ in vitro [53] and in vivo [54] and directly stimulate of adenylyl cyclase; at the same time, it prevents $G_{s\alpha}$ activation by G protein–coupled receptors, such as the bradykinin receptor, and reduces cAMP accumulation [54]. An increase in intracellular Ca^{2+}, such as occurs following EGFR activation, can stimulate cAMP phosphodiesterases, inhibit adenylyl cyclases, and inhibit vasopressin-induced renal accumulation of cAMP [55]. EGFR activation induces the generation of prostaglandin E_2 (PGE_2) in the collecting ducts and PGE_2 inhibits vasopressin-induced cAMP accumulation predominantly through stimulation of calcium-dependent phosphodiesterases and to a lesser extent by inhibition of the calcium inhibitable adenylate cyclase type 6 [56, 57]. EGF has been reported to down-regulate the expression of vasopressin V2 receptors in vivo [26] and to inhibit the hydrosmotic effect of vasopressin in isolated perfused rabbit cortical collecting tubules by a mechanism linked to PKC activation [58]. Finally, a number of tyrosine kinase inhibitors, such as genistein, erbstatin, tyrphostin B44, and herbimycin, have been reported to potentiate adrenergic or forskolin-induced cAMP accumulation in rat pinealocytes probably through inhibition of a cAMP phosphodiesterase [59].

CONCLUSION

The administration of EGFR tyrosine kinase inhibitors had unexpected effects on the development of PKD in the PCK rat, possibly due to stimulation of cAMP accumulation in collecting ducts with attendant proliferation and Cl^-/fluid secretion. The relevance of these observations to the treatment of human ARPKD or ADPKD remains uncertain and deserves further study.

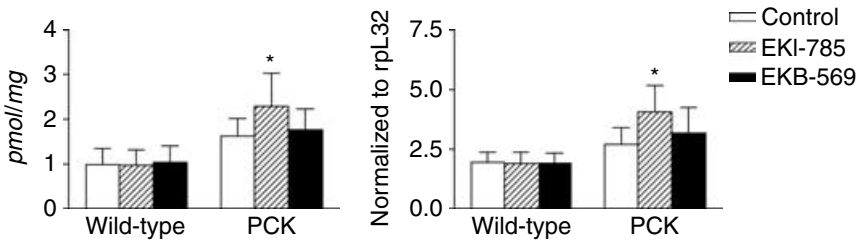

Fig. 3. Renal levels of adenosine 3′,5′-cyclic monophosphate (cAMP) and vasopressin V2 receptor mRNA in male control PCK rats and rats treated intraperitoneally with EKI-785 or EKB-569. *Significant difference ($P < 0.05$) compared to control.

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